METHOD FOR THE RECOMBINANT PRODUCTION OF 1,3-PROPANEDIOL

Related Applications

MF 01/12/05

The present application claims priority to the ~~is a continuation-in-part application of~~ United States Provisional Application 60/030,601 filed November 13, 1996, hereby incorporated herein in its entirety.

Field of Invention

The present invention relates to the field of molecular biology and specifically to improved methods for the production of 1,3-propanediol in host cells. In particular, the present invention describes components of gene clusters associated with 1,3-propanediol production in host cells, including protein X, and protein 1, protein 2 and protein 3. More specifically the present invention describes the expression of cloned genes encoding protein X , protein 1, protein 2 and protein 3, either separately or together, for the enhanced production of 1,3-propanediol in host cells.

Background

1,3-Propanediol is a monomer having potential utility in the production of polyester fibers and the manufacture of polyurethanes and cyclic compounds.

A variety of chemical routes to 1,3-propanediol are known. For example ethylene oxide may be converted to 1,3-propanediol over a catalyst in the presence of phosphine, water, carbon monoxide, hydrogen and an acid, by the catalytic solution phase hydration of acrolein followed by reduction, or from hydrocarbons such as glycerol, reacted in the presence of carbon monoxide and hydrogen over catalysts having atoms from group VIII of the periodic table. Although it is possible to generate 1,3-propanediol by these methods, they are expensive and generate waste streams containing environmental pollutants.

Suk B1

It has been known for over a century that 1,3-propanediol can be produced from the fermentation of glycerol. Bacterial strains able to produce 1,3-propanediol have been found, for example, in the groups *Citrobacter*, *Clostridium*, *Enterobacter*, *Ilyobacter*, *Klebsiella*, *Lactobacillus*, and *Pelobacter*. In each case studied, glycerol is converted to 1,3-propanediol in a two step, enzyme catalyzed reaction sequence. In the first step, a dehydratase catalyzes the conversion of glycerol to 3-hydroxypropionaldehyde (3-HP) and water (Equation 1). In the second step, 3-HP is reduced to 1,3-propanediol by a NAD^+-linked oxidoreductase (Equation 2).

$$\text{Glycerol} \rightarrow \text{3-HP} + H_2O \qquad \text{(Equation 1)}$$

$$\text{3-HP} + NADH + H^+ \rightarrow \text{1,3-Propanediol} + NAD^+ \qquad \text{(Equation 2)}$$

The 1,3-propanediol is not metabolized further and, as a result, accumulates in high concentration in the media. The overall reaction consumes a reducing equivalent in the form of a cofactor, reduced b-nicotinamide adenine dinucleotide (NADH), which is oxidized to nicotinamide adenine dinucleotide (NAD^+).